

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 June 2002 (20.06.2002)

PCT

(10) International Publication Number
WO 02/48362 A2

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/47, A01K 67/027, C07K 16/18, G01N 33/50,
C12Q 1/68, A61K 39/395

(74) Agents: HAMLET-COX, Diana et al.; Incyte Genomics,
Inc., 3160 Porter Drive, Palo Alto, CA 94304 (US).

(21) International Application Number: PCT/US01/43956

(22) International Filing Date:
14 November 2001 (14.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/249,407 15 November 2000 (15.11.2000) US

(71) Applicant (for all designated States except US): **INCYTE
GENOMICS, INC.** [US/US]; 3160 Porter Drive, Palo
Alto, CA 94304 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **RAMKUMAR,
Jayalaxmi** [IN/US]; 34359 Maybird Circle, Fremont, CA
94555 (US). **ARVIZU, Chandra** [US/US]; 490 Sherwood
Way #1, Menlo Park, CA 94025 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
TG).

Published:

— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: EMBRYOGENESIS ASSOCIATED PROTEINS

(57) Abstract: The invention provides human embryogenesis associated proteins (EMBRY) and polynucleotides which identify and encode EMBRY. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of EMBRY.

WO 02/48362 A2

EMBRYOGENESIS ASSOCIATED PROTEINS

TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of embryogenesis associated proteins and to the use of these sequences in the diagnosis, treatment, and prevention of reproductive disorders, autoimmune/inflammatory disorders, and disorders of the placenta, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of embryogenesis associated proteins.

BACKGROUND OF THE INVENTION

Mammalian embryogenesis is a process which encompasses the first few weeks of development following conception. During this period, embryogenesis proceeds from a single fertilized egg to the formation of the three embryonic tissues, then to an embryo which has most of its internal organs and all of its external features.

The normal course of mammalian embryogenesis depends on the correct temporal and spatial regulation of a large number of genes and tissues. These regulation processes have been intensely studied in mouse. An essential process that is still poorly understood is the activation of the embryonic genome after fertilization. As mouse oocytes grow, they accumulate transcripts that are either translated directly into proteins or stored for later activation by regulated polyadenylation. During subsequent meiotic maturation and ovulation, the maternal genome is transcriptionally inert, and most maternal transcripts are deadenylated and/or degraded prior to, or together with, the activation of the zygotic genes at the two-cell stage (Stutz, A. et al. (1998) *Genes Dev.* 12:2535-2548). The maternal to embryonic transition involves the degradation of oocyte, but not zygotic transcripts, the activation of the embryonic genome, and the induction of cell cycle progression to accommodate early development.

MATER (Maternal Antigen That Embryos Require) was initially identified as a target of antibodies from mice with ovarian immunity (Tong, Z.-B. and L.M. Nelson (1999) *Endocrinology* 140:3720-3726). Expression of the gene encoding MATER is restricted to the oocyte, making it one of a limited number of known maternal-effect genes in mammals (Tong, Z.-B. et al. (2000) *Mamm. Genome* 11:281-287). The MATER protein is required for embryonic development beyond two cells, based upon preliminary results from mice in which this gene has been inactivated. The 1111-amino acid MATER protein contains a hydrophilic repeat region in the amino terminus, and a region containing 14 leucine-rich repeats in the carboxyl terminus. These repeats resemble the sequence found in porcine ribonuclease inhibitor that is critical for protein-protein interactions.

The degradation of maternal transcripts during meiotic maturation and ovulation may involve

the activation of a ribonuclease just prior to ovulation. Thus the function of MATER may be to bind to the maternal ribonuclease and prevent degradation of zygotic transcripts (Tong (2000) *supra*). In addition to its role in oocyte development and embryogenesis, MATER may also be relevant to the pathogenesis of ovarian immunity, as it is a target of autoantibodies in mice with autoimmune

5 oophoritis (Tong (1999) *supra*).

Many other genes are involved in subsequent stages of embryogenesis. After fertilization, the oocyte is guided by fimbria at the distal end of each fallopian tube into and through the fallopian tube and thence into the uterus. Changes in the uterine endometrium prepare the tissue to support the implantation and embryonic development of a fertilized ovum. Several stages of division have
10 occurred before the dividing ovum, now a blastocyst with about 100 cells, enters the uterus. Upon reaching the uterus, the developing blastocyst usually remains in the uterine cavity an additional two to four days before implanting in the endometrium, the inner lining of the uterus. Implantation results from the action of trophoblast cells that develop over the surface of the blastocyst. These cells secrete proteolytic enzymes that digest and liquefy the cells of the endometrium. The invasive
15 process is reviewed in Fisher and Damsky (1993; *Semin. Cell Biol.* 4:183-188) and Graham and Lala (1992; *Biochem. Cell Biol.* 70:867-874). Once implantation has taken place, the trophoblast and other sublying cells proliferate rapidly, forming the placenta and the various membranes of pregnancy. (See Guyton, A.C. (1991) *Textbook of Medical Physiology*, 8th ed., W.B. Saunders Company, Philadelphia PA, pp. 915-919.)

20 The placenta has an essential role in protecting and nourishing the developing fetus. In most species the syncytiotrophoblast layer is present on the outside of the placenta at the fetal-maternal interface. This is a continuous structure, one cell deep, formed by the fusion of the constituent trophoblast cells. The syncytiotrophoblast cells play important roles in maternal-fetal exchange, in tissue remodeling during fetal development, and in protecting the developing fetus from the maternal
25 immune response (Stoye, J.P. and J.M. Coffin (2000) *Nature* 403:715-717).

A gene called syncytin is the envelope gene of a human endogenous defective provirus. Syncytin is expressed in high levels in placenta, and more weakly in testis, but is not detected in any other tissues (Mi, S. et al. (2000) *Nature* 403:785-789). Syncytin expression in the placenta is restricted to the syncytiotrophoblasts. Since retroviral *env* proteins are often involved in promoting
30 cell fusion events, it was thought that syncytin might be involved in regulating the fusion of trophoblast cells into the syncytiotrophoblast layer. Experiments demonstrated that syncytin can mediate cell fusion *in vitro*, and that anti-syncytin antibodies can inhibit the fusion of placental cytotrophoblasts (Mi, *supra*). In addition, a conserved immunosuppressive domain present in retroviral envelope proteins, and found in syncytin at amino acid residues 373-397, might be involved
35 in preventing maternal immune responses against the developing embryo.

Syncytin may also be involved in regulating trophoblast invasiveness by inducing trophoblast fusion and terminal differentiation (Mi, *supra*). Insufficient trophoblast infiltration of the uterine wall is associated with placental disorders such as preeclampsia, or pregnancy induced hypertension, while uncontrolled trophoblast invasion is observed in choriocarcinoma and other gestational trophoblastic diseases. Thus syncytin function may be involved in these diseases.

The discovery of new embryogenesis associated proteins, and the polynucleotides encoding them, satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of reproductive disorders, autoimmune/inflammatory disorders, and disorders of the placenta, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of embryogenesis associated proteins.

SUMMARY OF THE INVENTION

The invention features purified polypeptides, embryogenesis associated proteins, referred to collectively as "EMBRY" and individually as "EMBRY-1" and "EMBRY-2." In one aspect, the invention provides an isolated polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-2.

The invention further provides an isolated polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-2. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:3-4.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting

of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

The invention further provides an isolated polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group

consisting of SEQ ID NO:3-4, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The method

5 comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the

10 amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at

15 least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and,

20 optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected

25 from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and a pharmaceutically acceptable excipient. In one embodiment, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-

30 2. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional EMBRY, comprising administering to a patient in need of such treatment the composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino

35 acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a

naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional EMBRY, comprising administering to a patient in need of such treatment the composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional EMBRY, comprising administering to a patient in need of such treatment the composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino

acid sequence selected from the group consisting of SEQ ID NO:1-2, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide selected from the group consisting of i) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, iii) a polynucleotide having a sequence complementary to i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide selected from the group consisting of i) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4, iii) a polynucleotide complementary to the polynucleotide of i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated

biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

BRIEF DESCRIPTION OF THE TABLES

5 Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the present invention.

Table 2 shows the GenBank identification number and annotation of the nearest GenBank homolog for polypeptides of the invention. The probability scores for the matches between each polypeptide and its homolog(s) are also shown.

10 Table 3 shows structural features of polypeptide sequences of the invention, including predicted motifs and domains, along with the methods, algorithms, and searchable databases used for analysis of the polypeptides.

Table 4 lists the cDNA and/or genomic DNA fragments which were used to assemble polynucleotide sequences of the invention, along with selected fragments of the polynucleotide
15 sequences.

Table 5 shows the representative cDNA library for polynucleotides of the invention.

Table 6 provides an appendix which describes the tissues and vectors used for construction of the cDNA libraries shown in Table 5.

20 Table 7 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood
25 that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an,"
30 and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same
35 meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"EMBRY" refers to the amino acid sequences of substantially purified EMBRY obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of EMBRY. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of EMBRY either by directly interacting with EMBRY or by acting on components of the biological pathway in which EMBRY participates.

An "allelic variant" is an alternative form of the gene encoding EMBRY. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding EMBRY include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as EMBRY or a polypeptide with at least one functional characteristic of EMBRY. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding EMBRY, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding EMBRY. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent EMBRY. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of EMBRY is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and

threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of EMBRY. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of EMBRY either by directly interacting with EMBRY or by acting on components of the biological pathway in which EMBRY participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')₂, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind EMBRY polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "aptamer" refers to a nucleic acid or oligonucleotide molecule that binds to a specific molecular target. Aptamers are derived from an *in vitro* evolutionary process (e.g., SELEX (Systematic Evolution of Ligands by EXponential Enrichment), described in U.S. Patent No. 5,270,163), which selects for target-specific aptamer sequences from large combinatorial libraries. Aptamer compositions may be double-stranded or single-stranded, and may include

deoxyribonucleotides, ribonucleotides, nucleotide derivatives, or other nucleotide-like molecules.

The nucleotide components of an aptamer may have modified sugar groups (e.g., the 2'-OH group of a ribonucleotide may be replaced by 2'-F or 2'-NH₂), which may improve a desired property, e.g., resistance to nucleases or longer lifetime in blood. Aptamers may be conjugated to other molecules, e.g., a high molecular weight carrier to slow clearance of the aptamer from the circulatory system. Aptamers may be specifically cross-linked to their cognate ligands, e.g., by photo-activation of a cross-linker. (See, e.g., Brody, E.N. and L. Gold (2000) J. Biotechnol. 74:5-13.)

The term "intramer" refers to an aptamer which is expressed in vivo. For example, a vaccinia virus-based RNA expression system has been used to express specific RNA aptamers at high levels in the cytoplasm of leukocytes (Blind, M. et al. (1999) Proc. Natl Acad. Sci. USA 96:3606-3610).

The term "spiegelmer" refers to an aptamer which includes L-DNA, L-RNA, or other left-handed nucleotide derivatives or nucleotide-like molecules. Aptamers containing left-handed nucleotides are resistant to degradation by naturally occurring enzymes, which normally act on substrates containing right-handed nucleotides.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic EMBRY, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide

or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding EMBRY or fragments of EMBRY may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be
 5 deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (Applied Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been
 10 assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least
 15 interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
20	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
	Asp	Asn, Glu
	Cys	Ala, Ser
25	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
30	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
35	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

40 Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of

the side chain.

A “deletion” refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term “derivative” refers to a chemically modified polynucleotide or polypeptide.

5 Chemical modifications of a polynucleotide can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

10 A “detectable label” refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

“Differential expression” refers to increased or upregulated; or decreased, downregulated, or absent gene or protein expression, determined by comparing at least two different samples. Such comparisons may be carried out between, for example, a treated and an untreated sample, or a
15 diseased and a normal sample.

“Exon shuffling” refers to the recombination of different coding regions (exons). Since an exon may represent a structural or functional domain of the encoded protein, new proteins may be assembled through the novel reassortment of stable substructures, thus allowing acceleration of the evolution of new protein functions.

20 A “fragment” is a unique portion of EMBRY or the polynucleotide encoding EMBRY which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least
25 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by
30 the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:3-4 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:3-4, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:3-4 is useful, for
35 example, in hybridization and amplification technologies and in analogous methods that distinguish

SEQ ID NO:3-4 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:3-4 and the region of SEQ ID NO:3-4 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-2 is encoded by a fragment of SEQ ID NO:3-4. A fragment of
5 SEQ ID NO:1-2 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-2. For example, a fragment of SEQ ID NO:1-2 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-2. The precise length of a fragment of SEQ ID NO:1-2 and the region of SEQ ID NO:1-2 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the
10 fragment.

A "full length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full length" polynucleotide sequence encodes a "full length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between
15 two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and
20 therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in
25 Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at
30 <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence
35 analysis programs including "blastn," that is used to align a known polynucleotide sequence with

other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

Reward for match: 1

Penalty for mismatch: -2

Open Gap: 5 and Extension Gap: 2 penalties

Gap x drop-off: 50

Expect: 10

Word Size: 11

Filter: on

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of

polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

- 5 Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

Matrix: BLOSUM62

- 10 *Open Gap: 11 and Extension Gap: 1 penalties*

Gap x drop-off: 50

Expect: 10

Word Size: 3

Filter: on

- 15 Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment
- 20 length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size and which contain all of the elements required for chromosome replication, segregation and maintenance.

- 25 The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

- "Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific
- 30 hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive
- 35 conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill

in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

5 Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating T_m and
10 conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS,
15 for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular
20 circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid
25 sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

30 The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect
35 cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of EMBRY which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of EMBRY which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of EMBRY. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of EMBRY.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an EMBRY may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of EMBRY.

"Probe" refers to nucleic acid sequences encoding EMBRY, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target

polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and

polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

5 A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have
10 been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a
15 vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription,
20 translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

25 An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing EMBRY,
30 nucleic acids encoding EMBRY, or fragments thereof may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or
35 synthetic binding composition. The interaction is dependent upon the presence of a particular

structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

5 The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

10 A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

15 A "transcript image" or "expression profile" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed cells" includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

25 A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection,

35

transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides will generally have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length of one of the polypeptides.

THE INVENTION

The invention is based on the discovery of new human embryogenesis associated proteins (EMBRY), the polynucleotides encoding EMBRY, and the use of these compositions for the diagnosis, treatment, or prevention of reproductive disorders, autoimmune/inflammatory disorders, and disorders of the placenta.

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide

sequences of the invention. Each polynucleotide and its corresponding polypeptide are correlated to a single Incyte project identification number (Incyte Project ID). Each polypeptide sequence is denoted by both a polypeptide sequence identification number (Polypeptide SEQ ID NO:) and an Incyte polypeptide sequence number (Incyte Polypeptide ID) as shown. Each polynucleotide sequence is denoted by both a polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and an Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) as shown.

Table 2 shows sequences with homology to the polypeptides of the invention as identified by BLAST analysis against the GenBank protein (genpept) database. Columns 1 and 2 show the polypeptide sequence identification number (Polypeptide SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for polypeptides of the invention. Column 3 shows the GenBank identification number (GenBank ID NO:) of the nearest GenBank homolog. Column 4 shows the probability scores for the matches between each polypeptide and its homolog(s). Column 5 shows the annotation of the GenBank homolog(s) along with relevant citations where applicable, all of which are expressly incorporated by reference herein.

Table 3 shows various structural features of the polypeptides of the invention. Columns 1 and 2 show the polypeptide sequence identification number (SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for each polypeptide of the invention. Column 3 shows the number of amino acid residues in each polypeptide. Column 4 shows potential phosphorylation sites, and column 5 shows potential glycosylation sites, as determined by the MOTIFS program of the GCG sequence analysis software package (Genetics Computer Group, Madison WI). Column 6 shows amino acid residues comprising signature sequences, domains, and motifs. Column 7 shows analytical methods for protein structure/function analysis and in some cases, searchable databases to which the analytical methods were applied.

Together, Tables 2 and 3 summarize the properties of polypeptides of the invention, and these properties establish that the claimed polypeptides are embryogenesis associated proteins. For example, SEQ ID NO:2 is 88% identical to human syncytin precursor (GenBank ID g7595266) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is $6.2e-266$, which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:2 also contains an ENV polypeptide (coat polypeptide) domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from additional BLAST analyses using the PRODOM and DOMO databases provide further corroborative evidence that SEQ ID NO:2 is a syncytin-related embryogenesis associated protein. SEQ ID NO:1 was analyzed and annotated in a similar manner. The algorithms and parameters for the analysis of SEQ ID NO:1-2 are described in Table 7.

As shown in Table 4, the full length polynucleotide sequences of the present invention were assembled using cDNA sequences or coding (exon) sequences derived from genomic DNA, or any combination of these two types of sequences. Columns 1 and 2 list the polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and the corresponding Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) for each polynucleotide of the invention. Column 3 shows the length of each polynucleotide sequence in basepairs. Column 4 lists fragments of the polynucleotide sequences which are useful, for example, in hybridization or amplification technologies that identify SEQ ID NO:3-4 or that distinguish between SEQ ID NO:3-4 and related polynucleotide sequences. Column 5 shows identification numbers corresponding to cDNA sequences, coding sequences (exons) predicted from genomic DNA, and/or sequence assemblages comprised of both cDNA and genomic DNA. These sequences were used to assemble the full length polynucleotide sequences of the invention. Columns 6 and 7 of Table 4 show the nucleotide start (5') and stop (3') positions of the cDNA and/or genomic sequences in column 5 relative to their respective full length sequences.

The identification numbers in Column 5 of Table 4 may refer specifically, for example, to Incyte cDNAs along with their corresponding cDNA libraries. For example, 4400124H1 is the identification number of an Incyte cDNA sequence, and TESTTUT03 is the cDNA library from which it is derived. Incyte cDNAs for which cDNA libraries are not indicated were derived from pooled cDNA libraries. Alternatively, the identification numbers in column 5 may refer to GenBank cDNAs or ESTs (e.g., g2912466) which contributed to the assembly of the full length polynucleotide sequences. In addition, the identification numbers in column 5 may identify sequences derived from the ENSEMBL (The Sanger Centre, Cambridge, UK) database (*i.e.*, those sequences including the designation "ENST"). Alternatively, the identification numbers in column 5 may be derived from the NCBI RefSeq Nucleotide Sequence Records Database (*i.e.*, those sequences including the designation "NM" or "NT") or the NCBI RefSeq Protein Sequence Records (*i.e.*, those sequences including the designation "NP"). Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an "exon stitching" algorithm. For example, FL_XXXXXX_N₁_N₂_YYYYY_N₃_N₄ represents a "stitched" sequence in which XXXXXX is the identification number of the cluster of sequences to which the algorithm was applied, and YYYYY is the number of the prediction generated by the algorithm, and N_{1,2,3...}, if present, represent specific exons that may have been manually edited during analysis (See Example V). Alternatively, the identification numbers in column 5 may refer to assemblages of exons brought together by an "exon-stretching" algorithm. For example, FLXXXXXX_gAAAAA_gBBBBB_1_N is the identification number of a "stretched" sequence, with XXXXXX being the Incyte project identification number, gAAAAA being the GenBank identification number of the human genomic sequence to which the

“exon-stretching” algorithm was applied, *gBBBBB* being the GenBank identification number or NCBI RefSeq identification number of the nearest GenBank protein homolog, and *N* referring to specific exons (See Example V). In instances where a RefSeq sequence was used as a protein homolog for the “exon-stretching” algorithm, a RefSeq identifier (denoted by “NM,” “NP,” or “NT”) may be used in place of the GenBank identifier (*i.e.*, *gBBBBB*).

Alternatively, a prefix identifies component sequences that were hand-edited, predicted from genomic DNA sequences, or derived from a combination of sequence analysis methods. The following Table lists examples of component sequence prefixes and corresponding sequence analysis methods associated with the prefixes (see Example IV and Example V).

Prefix	Type of analysis and/or examples of programs
GNN, GFG, ENST	Exon prediction from genomic sequences using, for example, GENSCAN (Stanford University, CA, USA) or FGENES (Computer Genomics Group, The Sanger Centre, Cambridge, UK).
GBI	Hand-edited analysis of genomic sequences.
FL	Stitched or stretched genomic sequences (see Example V).
INCY	Full length transcript and exon prediction from mapping of EST sequences to the genome. Genomic location and EST composition data are combined to predict the exons and resulting transcript.

In some cases, Incyte cDNA coverage redundant with the sequence coverage shown in column 5 was obtained to confirm the final consensus polynucleotide sequence, but the relevant Incyte cDNA identification numbers are not shown.

Table 5 shows the representative cDNA libraries for those full length polynucleotide sequences which were assembled using Incyte cDNA sequences. The representative cDNA library is the Incyte cDNA library which is most frequently represented by the Incyte cDNA sequences which were used to assemble and confirm the above polynucleotide sequences. The tissues and vectors which were used to construct the cDNA libraries shown in Table 5 are described in Table 6.

The invention also encompasses EMBRY variants. A preferred EMBRY variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the EMBRY amino acid sequence, and which contains at least one functional or structural characteristic of EMBRY.

The invention also encompasses polynucleotides which encode EMBRY. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:3-4, which encodes EMBRY. The polynucleotide sequences of SEQ ID NO:3-4, as presented in the Sequence Listing, embrace the equivalent RNA

sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding EMBRY. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding EMBRY. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:3-4 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:3-4. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of EMBRY.

In addition, or in the alternative, a polynucleotide variant of the invention is a splice variant of a polynucleotide sequence encoding EMBRY. A splice variant may have portions which have significant sequence identity to the polynucleotide sequence encoding EMBRY, but will generally have a greater or lesser number of polynucleotides due to additions or deletions of blocks of sequence arising from alternate splicing of exons during mRNA processing. A splice variant may have less than about 70%, or alternatively less than about 60%, or alternatively less than about 50% polynucleotide sequence identity to the polynucleotide sequence encoding EMBRY over its entire length; however, portions of the splice variant will have at least about 70%, or alternatively at least about 85%, or alternatively at least about 95%, or alternatively 100% polynucleotide sequence identity to portions of the polynucleotide sequence encoding EMBRY. Any one of the splice variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of EMBRY.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding EMBRY, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring EMBRY, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode EMBRY and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring EMBRY under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding EMBRY or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-

naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding EMBRY and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode EMBRY and EMBRY derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding EMBRY or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:3-4 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Applied Biosystems), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) *Short Protocols in Molecular Biology*, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) *Molecular Biology and Biotechnology*, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding EMBRY may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic

DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.)

Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids

5 Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve

10 unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 primer analysis software (National

15 Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include

20 sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary

25 sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer

30 controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode EMBRY may be cloned in recombinant DNA molecules that direct expression of EMBRY, or fragments or functional equivalents thereof, in appropriate host cells. Due to the

35 inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same

or a functionally equivalent amino acid sequence may be produced and used to express EMBRY.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter EMBRY-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA
5 shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such
10 as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent No. 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of EMBRY, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of
15 gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random
20 point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding EMBRY may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucleic Acids Symp. Ser. 7:215-223; and Horn, T. et al. (1980) Nucleic Acids Symp. Ser. 7:225-232.)
Alternatively, EMBRY itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques.
30 (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Applied Biosystems). Additionally, the amino acid sequence of EMBRY, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or
35 a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, *supra*, pp. 28-53.)

5 In order to express a biologically active EMBRY, the nucleotide sequences encoding EMBRY or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in
10 polynucleotide sequences encoding EMBRY. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding EMBRY. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding EMBRY and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no
15 additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the
20 particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding EMBRY and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques,
25 and *in vivo* genetic recombination. (See, e.g., Sambrook, J. et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences
30 encoding EMBRY. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or
35 pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, *supra*; Ausubel, *supra*; Van Heeke,

G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; Engelhard, E.K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3224-3227; Sandig, V. et al. (1996) *Hum. Gene Ther.* 7:1937-1945; Takamatsu, N. (1987) *EMBO J.* 6:307-311; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) *Cancer Gen. Ther.* 5(6):350-356; Yu, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(13):6340-6344; Buller, R.M. et al. (1985) *Nature* 317(6040):813-815; McGregor, D.P. et al. (1994) *Mol. Immunol.* 31(3):219-226; and Verma, I.M. and N. Somia (1997) *Nature* 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding EMBRY. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding EMBRY can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPT1 plasmid (Life Technologies). Ligation of sequences encoding EMBRY into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509.) When large quantities of EMBRY are needed, e.g. for the production of antibodies, vectors which direct high level expression of EMBRY may be used. For example, vectors containing the strong, inducible SP6 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of EMBRY. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast *Saccharomyces cerevisiae* or *Pichia pastoris*. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) *Methods Enzymol.* 153:516-544; and Scorer, C.A. et al. (1994) *Bio/Technology* 12:181-184.)

Plant systems may also be used for expression of EMBRY. Transcription of sequences encoding EMBRY may be driven by viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al.

(1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.)

These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

5 In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding EMBRY may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses EMBRY in host cells. (See, e.g., Logan, J. and T. Shenk
10 (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are
15 constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of EMBRY in cell lines is preferred. For example, sequences encoding EMBRY can be transformed
20 into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which
25 successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk⁻* and *apr⁻* cells, respectively. (See, e.g., Wigler, M. et
30 al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g.,
Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981)
35 J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which

alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech), β glucuronidase and its substrate β -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to
5 quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding EMBRY is inserted within a marker gene sequence, transformed cells containing
10 sequences encoding EMBRY can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding EMBRY under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding EMBRY and that
15 express EMBRY may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of EMBRY using either
20 specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on EMBRY is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See,
25 e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and
30 may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding EMBRY include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding EMBRY, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available,
35 and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase

such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding EMBRY may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode EMBRY may be designed to contain signal sequences which direct secretion of EMBRY through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding EMBRY may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric EMBRY protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of EMBRY activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the EMBRY encoding sequence and the heterologous protein sequence, so that EMBRY may be cleaved away from the heterologous moiety following

purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

5 In a further embodiment of the invention, synthesis of radiolabeled EMBRY may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, ³⁵S-methionine.

10 EMBRY of the present invention or fragments thereof may be used to screen for compounds that specifically bind to EMBRY. At least one and up to a plurality of test compounds may be screened for specific binding to EMBRY. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

15 In one embodiment, the compound thus identified is closely related to the natural ligand of EMBRY, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, e.g., Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which EMBRY binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express EMBRY, either as a secreted
20 protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing EMBRY or cell membrane fractions which contain EMBRY are then contacted with a test compound and binding, stimulation, or inhibition of activity of either EMBRY or the compound is analyzed.

25 An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with EMBRY, either in solution or affixed to a solid support, and detecting the binding of EMBRY to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical
30 libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

EMBRY of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of EMBRY. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for
35 EMBRY activity, wherein EMBRY is combined with at least one test compound, and the activity of

EMBRY in the presence of a test compound is compared with the activity of EMBRY in the absence of the test compound. A change in the activity of EMBRY in the presence of the test compound is indicative of a compound that modulates the activity of EMBRY. Alternatively, a test compound is combined with an in vitro or cell-free system comprising EMBRY under conditions suitable for EMBRY activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of EMBRY may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding EMBRY or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding EMBRY may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding EMBRY can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding EMBRY is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease.

Alternatively, a mammal inbred to overexpress EMBRY, e.g., by secreting EMBRY in its milk, may

also serve as a convenient source of that protein (Janne, J. et al. (1998) *Biotechnol. Annu. Rev.* 4:55-74).

THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists
 5 between regions of EMBRY and embryogenesis associated proteins. In addition, the expression of EMBRY is closely associated with prostate tissue and T cells. Therefore, EMBRY appears to play a role in reproductive disorders, autoimmune/inflammatory disorders, and disorders of the placenta. In the treatment of disorders associated with increased EMBRY expression or activity, it is desirable to decrease the expression or activity of EMBRY. In the treatment of disorders associated with
 10 decreased EMBRY expression or activity, it is desirable to increase the expression or activity of EMBRY.

Therefore, in one embodiment, EMBRY or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EMBRY. Examples of such disorders include, but are not limited to, a reproductive
 15 disorder such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, ectopic pregnancy, teratogenesis; cancer of the breast, fibrocystic breast disease, galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology,
 20 cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, gynecomastia, hypergonadotropic and hypogonadotropic hypogonadism, pseudohermaphroditism, azoospermia, premature ovarian failure, acrosin deficiency, delayed puberty, retrograde ejaculation and anejaculation, haemangioblastomas, cystsphaeochromocytomas, paraganglioma, cystadenomas of the epididymis, and endolymphatic sac
 25 tumours; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), autoimmune oophoritis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis,
 30 dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid
 35 arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus,

systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a disorder of the placenta, such as preeclampsia, choriocarcinoma, abruptio placentae, placenta previa, placental or maternal floor infarction, placenta accreta, increta, and percreta, extrachorial placentas, chorangioma, chorangiosis, chronic villitis, placental villous edema, widespread fibrosis of the terminal villi, intervillous thrombi, hemorrhagic endovasculitis, erythroblastosis fetalis, and nonimmune fetal hydrops.

In another embodiment, a vector capable of expressing EMBRY or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EMBRY including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified EMBRY in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EMBRY including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of EMBRY may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of EMBRY including, but not limited to, those listed above.

In a further embodiment, an antagonist of EMBRY may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of EMBRY. Examples of such disorders include, but are not limited to, those reproductive disorders, autoimmune/inflammatory disorders, and disorders of the placenta described above. In one aspect, an antibody which specifically binds EMBRY may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express EMBRY.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding EMBRY may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of EMBRY including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of EMBRY may be produced using methods which are generally known in the art. In particular, purified EMBRY may be used to produce antibodies or to screen libraries of

pharmaceutical agents to identify those which specifically bind EMBRY. Antibodies to EMBRY may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with EMBRY or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to EMBRY have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of EMBRY amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to EMBRY may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce EMBRY-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as

disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for EMBRY may also be generated. For example, such fragments include, but are not limited to, $F(ab')_2$ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the $F(ab)_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between EMBRY and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering EMBRY epitopes is generally used, but a competitive binding assay may also be employed (Pound, *supra*).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for EMBRY. Affinity is expressed as an association constant, K_a , which is defined as the molar concentration of EMBRY-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The K_a determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple EMBRY epitopes, represents the average affinity, or avidity, of the antibodies for EMBRY. The K_a determined for a preparation of monoclonal antibodies, which are monospecific for a particular EMBRY epitope, represents a true measure of affinity. High-affinity antibody preparations with K_a ranging from about 10^9 to 10^{12} L/mole are preferred for use in immunoassays in which the EMBRY-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with K_a ranging from about 10^6 to 10^7 L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of EMBRY, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of EMBRY-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity,

and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding EMBRY, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding EMBRY. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding EMBRY. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding EMBRY may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) *Science* 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) *Science* 270:475-480; Bordignon, C. et al. (1995) *Science* 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) *Cell* 75:207-216; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:643-666; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) *Science* 270:404-410; Verma, I.M. and N. Somia (1997) *Nature* 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D.

(1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in EMBRY expression or regulation causes disease, the expression of EMBRY from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in EMBRY are treated by constructing mammalian expression vectors encoding EMBRY and introducing these vectors by mechanical means into EMBRY-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of EMBRY include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX, PCR2-TOPOTA vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). EMBRY may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or β -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) Proc. Natl. Acad. Sci. USA 89:5547-5551; Gossen, M. et al. (1995) Science 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, supra), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding EMBRY from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with

respect to EMBRY expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding EMBRY under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc. Natl. Acad. Sci. USA 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R. et al. (1998) J. Virol. 72:9873-9880). U.S. Patent No. 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4⁺ T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding EMBRY to cells which have one or more genetic abnormalities with respect to the expression of EMBRY. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent No. 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544 and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding EMBRY to target cells which have one or more genetic abnormalities with respect to the expression of EMBRY. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing EMBRY to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with

ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) *Exp. Eye Res.* 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent No. 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent No. 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) *J. Virol.* 73:519-532 and Xu, H. et al. (1994) *Dev. Biol.* 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding EMBRY to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) *Curr. Opin. Biotechnol.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for EMBRY into the alphavirus genome in place of the capsid-coding region results in the production of a large number of EMBRY-coding RNAs and the synthesis of high levels of EMBRY in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of EMBRY into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly,

inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.L. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding EMBRY.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding EMBRY. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding EMBRY.

Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming

- 5 oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased EMBRY expression or activity, a compound which specifically inhibits expression of the
- 10 polynucleotide encoding EMBRY may be therapeutically useful, and in the treatment of disorders associated with decreased EMBRY expression or activity, a compound which specifically promotes expression of the polynucleotide encoding EMBRY may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method

15 commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a

20 polynucleotide encoding EMBRY is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an *in vitro* cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding EMBRY are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence

25 complementary to the sequence of the polynucleotide encoding EMBRY. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound

30 effective in altering expression of a specific polynucleotide can be carried out, for example, using a *Schizosaccharomyces pombe* gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides

35 (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides)

for antisense activity against a specific polynucleotide sequence (Bruice, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruice, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of EMBRY, antibodies to EMBRY, and mimetics, agonists, antagonists, or inhibitors of EMBRY.

The compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

Specialized forms of compositions may be prepared for direct intracellular delivery of macromolecules comprising EMBRY or fragments thereof. For example, liposome preparations

containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, EMBRY or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example EMBRY or fragments thereof, antibodies of EMBRY, and agonists, antagonists or inhibitors of EMBRY, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the LD_{50}/ED_{50} ratio. Compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind EMBRY may be used for the diagnosis of disorders characterized by expression of EMBRY, or in assays to monitor patients being treated with EMBRY or agonists, antagonists, or inhibitors of EMBRY. Antibodies useful for
5 diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for EMBRY include methods which utilize the antibody and a label to detect EMBRY in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in
10 the art and may be used.

A variety of protocols for measuring EMBRY, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of EMBRY expression. Normal or standard values for EMBRY expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibodies to
15 EMBRY under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of EMBRY expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding EMBRY may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of EMBRY may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess
20 expression of EMBRY, and to monitor regulation of EMBRY levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding EMBRY or closely related molecules may be used to identify nucleic acid sequences which encode EMBRY. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region,
30 e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding EMBRY, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the EMBRY encoding sequences. The hybridization probes of the subject
35 invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:3-4 or from

genomic sequences including promoters, enhancers, and introns of the EMBRY gene.

Means for producing specific hybridization probes for DNAs encoding EMBRY include the cloning of polynucleotide sequences encoding EMBRY or EMBRY derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may
 5 be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding EMBRY may be used for the diagnosis of disorders
 10 associated with expression of EMBRY. Examples of such disorders include, but are not limited to, a reproductive disorder such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, ectopic pregnancy, teratogenesis; cancer of the breast,
 15 fibrocystic breast disease, galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis; cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, gynecomastia, hypergonadotropic and hypogonadotropic hypogonadism, pseudohermaphroditism, azoospermia, premature ovarian failure, acrosin deficiency, delayed puberty, retrograde ejaculation and anejaculation, haemangioblastomas,
 20 cystsphaeochromocytomas, paraganglioma, cystadenomas of the epididymis, and endolymphatic sac tumours; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), autoimmune
 25 oophoritis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation,
 30 osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and a disorder of the placenta, such as
 35 preeclampsia, choriocarcinoma, abruptio placentae, placenta previa, placental or maternal floor

infarction, placenta accreta, increta, and percreta, extrachorial placentas, chorangioma, chorangiosis, chronic villitis, placental villous edema, widespread fibrosis of the terminal villi, intervillous thrombi, hemorrhagic endovasculitis, erythroblastosis fetalis, and nonimmune fetal hydrops. The polynucleotide sequences encoding EMBRY may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered EMBRY expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding EMBRY may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding EMBRY may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding EMBRY in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of EMBRY, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding EMBRY, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals

to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding EMBRY may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding EMBRY, or a fragment of a polynucleotide complementary to the polynucleotide encoding EMBRY, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding EMBRY may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding EMBRY are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of EMBRY include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the

polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described below. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene
5 function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly
10 effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, EMBRY, fragments of EMBRY, or antibodies specific for EMBRY may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described
15 above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at
20 a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present
25 invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

30 Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and
35 toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson

(2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from

biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by
5 comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for EMBRY to quantify the levels of EMBRY expression. In one embodiment, the antibodies are used as elements on a
10 microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoz, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at
15 each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be
20 useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological
25 sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing
30 the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of
35 protein recognized by the antibodies is quantified. The amount of protein in the treated biological

sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed.

(1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding EMBRY may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, for example, Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding EMBRY on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is

valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, EMBRY, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between EMBRY and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with EMBRY, or fragments thereof, and washed. Bound EMBRY is then detected by methods well known in the art. Purified EMBRY can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding EMBRY specifically compete with a test compound for binding EMBRY. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with EMBRY.

In additional embodiments, the nucleotide sequences which encode EMBRY may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications and publications, mentioned above and below, including U.S. Ser. No. 60/249,407, are expressly incorporated by reference herein.

EXAMPLES

I. Construction of cDNA Libraries

Incyte cDNAs were derived from cDNA libraries described in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA) and shown in Table 4, column 5. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A)+ RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, *supra*, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), PCR2-TOPOTA plasmid (Invitrogen), PCMV-ICIS plasmid (Stratagene), pIGEN (Incyte Genomics, Palo Alto CA), or pINCY (Incyte Genomics), or derivatives thereof. Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 α , DH10B, or ElectroMAX DH10B from Life Technologies.

II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by *in vivo* excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an

AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (LabSystems Oy, Helsinki, Finland).

III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Applied Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VIII.

The polynucleotide sequences derived from Incyte cDNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations thereof were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM; PROTEOME databases with sequences from Homo sapiens, Rattus norvegicus, Mus musculus, Caenorhabditis elegans, Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Candida albicans (Incyte Genomics, Palo Alto CA); and hidden Markov model (HMM)-based protein family databases such as PFAM. (HMM is a probabilistic approach which analyzes consensus primary structures of gene families. See, for example, Eddy,

S.R. (1996) *Curr. Opin. Struct. Biol.* 6:361-365.) The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were assembled to produce full length polynucleotide sequences. Alternatively, GenBank cDNAs, GenBank ESTs, stitched sequences, stretched sequences, or Genscan-predicted coding sequences (see Examples IV and V) were used to extend Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Alternatively, a polypeptide of the invention may begin at any of the methionine residues of the full length translated polypeptide. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein databases (genpept), SwissProt, the PROTEOME databases, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 7 summarizes the tools, programs, and algorithms used for the analysis and assembly of Incyte cDNA and full length sequences and provides applicable descriptions, references, and threshold parameters. The first column of Table 7 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score or the lower the probability value, the greater the identity between two sequences).

The programs described above for the assembly and analysis of full length polynucleotide and polypeptide sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:3-4. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies are described in Table 4, column 4.

IV. Identification and Editing of Coding Sequences from Genomic DNA

Putative embryogenesis associated proteins were initially identified by running the Genscan gene identification program against public genomic sequence databases (e.g., gbpri and gbhtg). Genscan is a general-purpose gene identification program which analyzes genomic DNA sequences from a variety of organisms (See Burge, C. and S. Karlin (1997) *J. Mol. Biol.* 268:78-94, and Burge,

C. and S. Karlin (1998) *Curr. Opin. Struct. Biol.* 8:346-354). The program concatenates predicted exons to form an assembled cDNA sequence extending from a methionine to a stop codon. The output of Genscan is a FASTA database of polynucleotide and polypeptide sequences. The maximum range of sequence for Genscan to analyze at once was set to 30 kb. To determine which of these

5 Genscan predicted cDNA sequences encode embryogenesis associated proteins, the encoded polypeptides were analyzed by querying against PFAM models for embryogenesis associated proteins. Potential embryogenesis associated proteins were also identified by homology to Incyte cDNA sequences that had been annotated as embryogenesis associated proteins. These selected Genscan-predicted sequences were then compared by BLAST analysis to the genpept and gbpi

10 public databases. Where necessary, the Genscan-predicted sequences were then edited by comparison to the top BLAST hit from genpept to correct errors in the sequence predicted by Genscan, such as extra or omitted exons. BLAST analysis was also used to find any Incyte cDNA or public cDNA coverage of the Genscan-predicted sequences, thus providing evidence for transcription. When Incyte cDNA coverage was available, this information was used to correct or confirm the

15 Genscan predicted sequence. Full length polynucleotide sequences were obtained by assembling Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the assembly process described in Example III. Alternatively, full length polynucleotide sequences were derived entirely from edited or unedited Genscan-predicted coding sequences.

V. Assembly of Genomic Sequence Data with cDNA Sequence Data

20 "Stitched" Sequences

Partial cDNA sequences were extended with exons predicted by the Genscan gene identification program described in Example IV. Partial cDNAs assembled as described in Example III were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm

25 based on graph theory and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or extended to create a full length sequence. Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified, and intervals thus identified were considered to be equivalent by transitivity. For example, if an interval was present on a cDNA and two genomic

30 sequences, then all three intervals were considered to be equivalent. This process allows unrelated but consecutive genomic sequences to be brought together, bridged by cDNA sequence. Intervals thus identified were then "stitched" together by the stitching algorithm in the order that they appear along their parent sequences to generate the longest possible sequence, as well as sequence variants. Linkages between intervals which proceed along one type of parent sequence (cDNA to cDNA or

35 genomic sequence to genomic sequence) were given preference over linkages which change parent

type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbprl public databases. Incorrect exons predicted by Genscan were corrected by comparison to the top BLAST hit from genpept. Sequences were further extended with additional cDNA sequences, or by inspection of genomic DNA, when necessary.

5 "Stretched" Sequences

Partial DNA sequences were extended to full length with an algorithm based on BLAST analysis. First, partial cDNAs assembled as described in Example III were queried against public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases using the BLAST program. The nearest GenBank protein homolog was then compared by BLAST
10 analysis to either Incyte cDNA sequences or GenScan exon predicted sequences described in Example IV. A chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. Insertions or deletions may occur in the chimeric protein with respect to the original GenBank protein homolog. The GenBank protein homolog, the chimeric protein, or both were used as probes to search for
15 homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore "stretched" or extended by the addition of homologous genomic sequences. The resultant stretched sequences were examined to determine whether it contained a complete gene.

VI. Chromosomal Mapping of EMBRY Encoding Polynucleotides

The sequences which were used to assemble SEQ ID NO:3-4 were compared with sequences
20 from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:3-4 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 7). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for
25 Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

Map locations are represented by ranges, or intervals, of human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-
30 arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and
35 other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site

(<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

VII. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel (1995) *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum \{length(Seq. 1), length(Seq. 2)\}}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

Alternatively, polynucleotide sequences encoding EMBRY are analyzed with respect to the tissue sources from which they were derived. For example, some full length sequences are assembled, at least in part, with overlapping Incyte cDNA sequences (see Example III). Each cDNA sequence is derived from a cDNA library constructed from a human tissue. Each human tissue is classified into one of the following organ/tissue categories: cardiovascular system; connective tissue; digestive system; embryonic structures; endocrine system; exocrine glands; genitalia, female;

genitalia, male; germ cells; hemic and immune system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs; skin; stomatognathic system; unclassified/mixed; or urinary tract. The number of libraries in each category is counted and divided by the total number of libraries across all categories. Similarly, each human tissue is classified into one of the following disease/condition categories: cancer, cell line, developmental, inflammation, neurological, trauma, cardiovascular, pooled, and other, and the number of libraries in each category is counted and divided by the total number of libraries across all categories. The resulting percentages reflect the tissue- and disease-specific expression of cDNA encoding EMBRY. cDNA sequences and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA).

10 VIII. Extension of EMBRY Encoding Polynucleotides

Full length polynucleotide sequences were also produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer was synthesized to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and 2-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 μ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 μ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the

concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, 5 digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham 10 Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase 15 (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted 20 with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, full length polynucleotide sequences are verified using the above procedure or are used to obtain 5' regulatory sequences using the above procedure along with oligonucleotides 25 designed for such extension, and an appropriate genomic library.

IX. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:3-4 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. 30 Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μ Ci of [γ -³²P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10⁷ 35 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of

human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

X. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, *supra.*), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schena (1999), *supra.*). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers.

Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645; Marshall, A. and J. Hodgson (1998) *Nat. Biotechnol.* 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)⁺ RNA is purified using the oligo-(dT) cellulose method. Each poly(A)⁺ RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/μl oligo-(dT) primer (21mer), 1X

first strand buffer, 0.03 units/ μ l RNase inhibitor, 500 μ M dATP, 500 μ M dGTP, 500 μ M dTTP, 40 μ M dCTP, 40 μ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)⁺ RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)⁺ RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14 μ l 5X SSC/0.2% SDS.

Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5 μ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in U.S. Patent No. 5,807,522, incorporated herein by reference. 1 μ l of the array element DNA, at an average concentration of 100 ng/ μ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60°C followed by washes in 0.2% SDS and distilled water as before.

Hybridization

Hybridization reactions contain 9 μ l of sample mixture consisting of 0.2 μ g each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65°C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm² coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 μ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60°C. The arrays are washed for 10 min at 45°C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45°C in a second wash buffer (0.1X SSC), and dried.

10 Detection

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a

linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

5 A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

XI. Complementary Polynucleotides

10 Sequences complementary to the EMBRY-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring EMBRY. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of EMBRY.

15 To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the EMBRY-encoding transcript.

XII. Expression of EMBRY

20 Expression and purification of EMBRY is achieved using bacterial or virus-based expression systems. For expression of EMBRY in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3).

25 Antibiotic resistant bacteria express EMBRY upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of EMBRY in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant *Autographica californica* nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding EMBRY by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect *Spodoptera frugiperda* (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther.

35

7:1937-1945.)

In most expression systems, EMBRY is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-

- 5 kilodalton enzyme from *Schistosoma japonicum*, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from EMBRY at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman
10 Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified EMBRY obtained by these methods can be used directly in the assays shown in Examples XVI and XVII, where applicable.

XIII. Functional Assays

- 15 EMBRY function is assessed by expressing the sequences encoding EMBRY at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include PCMV SPORT (Life Technologies) and PCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10 μ g of recombinant vector
20 are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2 μ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green
25 Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA
30 with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in
35 flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of EMBRY on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding EMBRY and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding EMBRY and other genes of interest can be analyzed by northern analysis or microarray techniques.

XIV. Production of EMBRY Specific Antibodies

EMBRY substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the EMBRY amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using Fmoc chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide and anti-EMBRY activity by, for example, binding the peptide or EMBRY to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XV. Purification of Naturally Occurring EMBRY Using Specific Antibodies

Naturally occurring or recombinant EMBRY is substantially purified by immunoaffinity chromatography using antibodies specific for EMBRY. An immunoaffinity column is constructed by covalently coupling anti-EMBRY antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing EMBRY are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of EMBRY (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/EMBRY binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such

as urea or thiocyanate ion), and EMBRY is collected.

XVI. Identification of Molecules Which Interact with EMBRY

EMBRY, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton, A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled EMBRY, washed, and any wells with labeled EMBRY complex are assayed. Data obtained using different concentrations of EMBRY are used to calculate values for the number, affinity, and association of EMBRY with the candidate molecules.

Alternatively, molecules interacting with EMBRY are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989) *Nature* 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

EMBRY may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S.

Patent No. 6,057,101).

XVII. Demonstration of EMBRY Activity

An assay for EMBRY-1 activity measures the effect of injected EMBRY-1 on the degradation of maternal transcripts. Procedures for oocyte collection from Swiss albino mice, injection, and culture are as described in Stutz, *supra*. A decrease in the degradation of maternal RNAs as compared to control oocytes is indicative of EMBRY-1 activity. In the alternative, EMBRY-1 activity is measured as the ability of purified EMBRY-1 to bind to RNase as measured by the assays described in Example XVI.

An assay for EMBRY-2 activity measures syncytium formation in COS cells transfected with an EMBRY-2 expression plasmid, using the two-component fusion assay described in Mi et al., *supra*. This assay takes advantage of the fact that human interleukin 12 (IL-12) is a heterodimer comprising subunits with molecular weights of 35 kD (p35) and 40 kD (p40). COS cells transfected with expression plasmids carrying the gene for p35, are mixed with COS cells cotransfected with expression plasmids carrying the genes for p40 and EMBRY-2. The level of IL-12 activity in the resulting conditioned medium corresponds to the activity of EMBRY-2 in this assay. Syncytium formation may also be measured by light microscopy (Mi et al., *supra*).

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific

embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Incyte Project ID	Polypeptide SEQ ID NO:	Incyte Polypeptide ID	Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID
7474830	1	7474830CD1	3	7474830CB1
7477736	2	7477736CD1	4	7477736CB1

Table 2

Polypeptide SEQ ID NO:	Incyte Polypeptide ID	GenBank ID NO:	Probability Score	GenBank Homolog
1	7474830CD1	g7595266	9.0e-288	[Mus musculus] maternal-antigen-that-embryos-require protein; MATER; ooplasm-specific protein; OPI Tong, Z.B. et al. (2000) Mater encodes a maternal protein in mice with a leucine-rich repeat domain homologous to porcine ribonuclease inhibitor. Mamm. Genome 11:281-287
2	7477736CD1	g6760401	6.2e-266	[Homo sapiens] syncytin precursor Mi, S. et al. (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature 403:785-789

Table 3

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
1	7474830CD1	1162	S1097 S1151 S148 S234 S292 S293 S314 S37 S38 S385 S506 S536 S548 S668 S77 S832 S857 S88 S911 S936 S98 T105 T162 T181 T218 T28 T370 T376 T473 T577 T642 T726 T756 T771 T86 Y121	N335 N457 N639 N912 N922	Leucine Rich Repeat: H1085-T1112, H744-R768, N800-K827, L829-P856, S857-L880, A886-L909, S914-R938, S943-A965, H1000-I1023, H1028-K1052 RIBONUCLEASE INHIBITOR REPEAT LEUCINEREPEAT 3DSTRUCTURE PLACENTAL RIBONUCLEASE/ANGIOGENIN RAI RI RECEPTOR PD017636: L861-L966, L975-L1080, Q747-A843 RECEPTOR ANGIOTENSIN/VASOPRESSIN AII/AVP VASOPRESSIN PD156095: V449-F638 ATP/GTP binding site (P-loop): G248-S255	HMER-PFAM
2	7477736CD1	542	S225 S477 S478 S51 T157 T301 T367 T390 T415 T88 Y52	N169 N208 N214 N234 N281 N409	Signal peptide: M1-T20 Transmembrane domain: L455-V473 ENV polypeptide (coat polypeptide) domain: C263-K484 PROTEIN POLYPEPTIDE ENVELOPE GLYCOPROTEIN ENV COAT PRECURSOR SIGNAL CONTAINS: TRANSMEMERANE PD000750: L164-Y502 TYPE C RETROVIRUS ENV POLYPEPTIDE: DM00268 P10269 348-528: N307-F469 DM00268 P07575 366-546: N307-F469 DM00268 P03399 364-544: H314-I468 DM00268 P51515 354-534: K316-F469	SPSCAN HMER HMER-PFAM BLAST- PRODOM BLAST-DOMO

Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragments	Sequence Fragments	5' Position	3' Position
3	7474830CB1	3489	1-720, 3070-3197, 992-1012, 1112-2456	FL7474830_g9211204_g5802698	1	3489
4	7477736CB1	2074	1-627, 1-884, 1312-1501	4400124H1 (TESTTUT03) 7199707H1 (LUNCFER04) 6121150H1 (BRAHNON05) g2912466 GBI.g9797187_000003.edit 4741292H1 (THYMNOR02) 4959790F6 (TLXMNOT05)	437 1342 867 1642 64 794 1	604 1864 1542 2074 1689 956 276

Table 5

Polynucleotide SEQ ID NO:	Incyte Project ID	Representative Library
3	7474830CB1	PROSTMC01
4	7477736CB1	TLYMNOT05

Table 6

Library	Vector	Library Description
PROSTMC01	pINCY	Library was constructed using polyA RNA isolated from diseased prostate tissue removed from a 55-year-old Caucasian male during a radical prostatectomy, regional lymph node excision, and prostate needle biopsy. Pathology indicated adenofibromatous hyperplasia. Pathology for the matched tumor tissue indicated adenocarcinoma, Gleason grade 5+4, forming a predominant mass involving the left side peripherally with extension into the right posterior superior region. The tumor invaded and perforated the capsule to involve periprostatic tissue in the left posterior superior region. The left inferior and superior posterior surgical margins were positive. The right and left seminal vesicles, bladder neck tissue (after re-excision), and multiple pelvic lymph nodes were negative for tumor. One (of 9) left pelvic lymph nodes was metastatically involved. The patient presented with elevated prostate specific antigen (PSA). Patient history included calculus of the kidney. Previous surgeries included an adenotonsillectomy. Patient medications included Khats claw, an herbal preparation. Family history included breast cancer in the mother; lung cancer in the father; and breast cancer in the sibling(s).
TLYMNOT05	pINCY	Library was constructed using RNA isolated from nonactivated Th2 cells. These cells were differentiated from umbilical cord CD4 T cells with IL-4 in the presence of anti-IL-12 antibodies and B7-transfected COS cells.

Table 7

Program	Description	Reference	Parameter Threshold
ABI/FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABI/PARACEL.FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value= 1.0E-3 or less Signal peptide hits: Score= 0 or greater

Table 7 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribskov, M. et al. (1988) CABIOS 4:61-66; Gribskov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score \geq GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Pfired	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
TMAP	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
TMHMMER	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. An isolated polypeptide selected from the group consisting of:

- 5 a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2,
- b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-2,
- 10 c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, and
- d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

2. An isolated polypeptide of claim 1 comprising an amino acid sequence selected from the

15 group consisting of SEQ ID NO:1-2.

3. An isolated polynucleotide encoding a polypeptide of claim 1.

4. An isolated polynucleotide encoding a polypeptide of claim 2.

20

5. An isolated polynucleotide of claim 4 comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4.

6. A recombinant polynucleotide comprising a promoter sequence operably linked to a

25 polynucleotide of claim 3.

7. A cell transformed with a recombinant polynucleotide of claim 6.

8. A transgenic organism comprising a recombinant polynucleotide of claim 6.

30

9. A method of producing a polypeptide of claim 1, the method comprising:

- a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and
- 35

- b) recovering the polypeptide so expressed.

10. A method of claim 9, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

5

11. An isolated antibody which specifically binds to a polypeptide of claim 1.

12. An isolated polynucleotide selected from the group consisting of:

10

- a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4,
b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:3-4,
c) a polynucleotide complementary to a polynucleotide of a),
15 d) a polynucleotide complementary to a polynucleotide of b), and
e) an RNA equivalent of a)-d).

15

13. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 12.

20

14. A method of detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 12, the method comprising:

25

- a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and
b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

30

15. A method of claim 14, wherein the probe comprises at least 60 contiguous nucleotides.

16. A method of detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 12, the method comprising:

35

- a) amplifying said target polynucleotide or fragment thereof using polymerase chain

- reaction amplification, and
- b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

5 17. A composition comprising a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

18. A composition of claim 17, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

10

19. A method for treating a disease or condition associated with decreased expression of functional EMBRY, comprising administering to a patient in need of such treatment the composition of claim 17.

15

20. A method of screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting agonist activity in the sample.

20

21. A composition comprising an agonist compound identified by a method of claim 20 and a pharmaceutically acceptable excipient.

22. A method for treating a disease or condition associated with decreased expression of functional EMBRY, comprising administering to a patient in need of such treatment a composition of claim 21.

25

23. A method of screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

30

24. A composition comprising an antagonist compound identified by a method of claim 23 and a pharmaceutically acceptable excipient.

35

25. A method for treating a disease or condition associated with overexpression of functional

EMBRY, comprising administering to a patient in need of such treatment a composition of claim 24.

26. A method of screening for a compound that specifically binds to the polypeptide of claim 1, the method comprising:

- a) combining the polypeptide of claim 1 with at least one test compound under suitable conditions, and
- b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a compound that specifically binds to the polypeptide of claim 1.

27. A method of screening for a compound that modulates the activity of the polypeptide of claim 1, the method comprising:

- a) combining the polypeptide of claim 1 with at least one test compound under conditions permissive for the activity of the polypeptide of claim 1,
- b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound, and
- c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change in the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide of claim 1.

28. A method of screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method comprising:

- a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,
- b) detecting altered expression of the target polynucleotide, and
- c) comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound.

29. A method of assessing toxicity of a test compound, the method comprising:

- a) treating a biological sample containing nucleic acids with the test compound,
- b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide of claim 12 under conditions whereby a specific hybridization complex is formed between said probe and a target

polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 12 or fragment thereof,

- c) quantifying the amount of hybridization complex, and
- d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

30. A diagnostic test for a condition or disease associated with the expression of EMBRY in

10 a biological sample; the method comprising:

- a) combining the biological sample with an antibody of claim 11, under conditions suitable for the antibody to bind the polypeptide and form an antibody:polypeptide complex, and
- b) detecting the complex, wherein the presence of the complex correlates with the presence of the polypeptide in the biological sample.

31. The antibody of claim 11, wherein the antibody is:

- a) a chimeric antibody,
- b) a single chain antibody,
- c) a Fab fragment,
- d) a F(ab')₂ fragment, or
- e) a humanized antibody.

32. A composition comprising an antibody of claim 11 and an acceptable excipient.

33. A method of diagnosing a condition or disease associated with the expression of EMBRY in a subject, comprising administering to said subject an effective amount of the composition of claim 32.

34. A composition of claim 32, wherein the antibody is labeled.

35. A method of diagnosing a condition or disease associated with the expression of EMBRY in a subject, comprising administering to said subject an effective amount of the composition of claim 34.

36. A method of preparing a polyclonal antibody with the specificity of the antibody of claim 11, the method comprising:

- a) immunizing an animal with a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or an immunogenic fragment thereof, under conditions to elicit an antibody response,
- b) isolating antibodies from said animal, and
- c) screening the isolated antibodies with the polypeptide, thereby identifying a polyclonal antibody which binds specifically to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

37. A polyclonal antibody produced by a method of claim 36.

38. A composition comprising the polyclonal antibody of claim 37 and a suitable carrier.

39. A method of making a monoclonal antibody with the specificity of the antibody of claim 11, the method comprising:

- a) immunizing an animal with a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:1-2, or an immunogenic fragment thereof, under conditions to elicit an antibody response,
- b) isolating antibody producing cells from the animal,
- c) fusing the antibody producing cells with immortalized cells to form monoclonal antibody-producing hybridoma cells,
- d) culturing the hybridoma cells, and
- e) isolating from the culture monoclonal antibody which binds specifically to a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

40. A monoclonal antibody produced by a method of claim 39.

41. A composition comprising the monoclonal antibody of claim 40 and a suitable carrier.

42. The antibody of claim 11, wherein the antibody is produced by screening a Fab expression library.

43. The antibody of claim 11, wherein the antibody is produced by screening a recombinant

immunoglobulin library.

44. A method of detecting a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2 in a sample, the method comprising:

- a) incubating the antibody of claim 11 with a sample under conditions to allow specific binding of the antibody and the polypeptide, and
- b) detecting specific binding, wherein specific binding indicates the presence of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2 in the sample.

45. A method of purifying a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2 from a sample, the method comprising:

- a) incubating the antibody of claim 11 with a sample under conditions to allow specific binding of the antibody and the polypeptide, and
- b) separating the antibody from the sample and obtaining the purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-2.

46. A microarray wherein at least one element of the microarray is a polynucleotide of claim

47. A method of generating an expression profile of a sample which contains polynucleotides, the method comprising:

- a) labeling the polynucleotides of the sample,
- b) contacting the elements of the microarray of claim 46 with the labeled polynucleotides of the sample under conditions suitable for the formation of a hybridization complex, and
- c) quantifying the expression of the polynucleotides in the sample.

48. An array comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate, wherein at least one of said nucleotide molecules comprises a first oligonucleotide or polynucleotide sequence specifically hybridizable with at least 30 contiguous nucleotides of a target polynucleotide, and wherein said target polynucleotide is a polynucleotide of claim 12.

49. An array of claim 48, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to at least 30 contiguous nucleotides of said target polynucleotide.

50. An array of claim 48, wherein said first oligonucleotide or polynucleotide sequence is
5 completely complementary to at least 60 contiguous nucleotides of said target polynucleotide.

51. An array of claim 48, wherein said first oligonucleotide or polynucleotide sequence is completely complementary to said target polynucleotide.

10 52. An array of claim 48, which is a microarray.

53. An array of claim 48, further comprising said target polynucleotide hybridized to a nucleotide molecule comprising said first oligonucleotide or polynucleotide sequence.

15 54. An array of claim 48, wherein a linker joins at least one of said nucleotide molecules to said solid substrate.

55. An array of claim 48, wherein each distinct physical location on the substrate contains multiple nucleotide molecules, and the multiple nucleotide molecules at any single distinct physical
20 location have the same sequence, and each distinct physical location on the substrate contains nucleotide molecules having a sequence which differs from the sequence of nucleotide molecules at another distinct physical location on the substrate.

25 56. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:1.

57. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2.

58. A polynucleotide of claim 12, comprising the polynucleotide sequence of SEQ ID NO:3.

30 59. A polynucleotide of claim 12, comprising the polynucleotide sequence of SEQ ID NO:4.

<110> INCYTE GENOMICS, INC.
 RAMKUMAR, Jayalaxmi
 ARVIZU, Chandra

<120> EMBRYOGENESIS ASSOCIATED PROTEINS

<130> PF-0842 PCT

<140> To Be Assigned

<141> Herewith

<150> 60/249,407

<151> 2000-11-15

<160> 4

<170> PERL Program

<210> 1

<211> 1162

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 7474830CD1

<400> 1

Met	Glu	Gly	Asp	Lys	Ser	Leu	Thr	Phe	Ser	Ser	Tyr	Gly	Leu	Gln	1	5	10	15
Trp	Cys	Leu	Tyr	Glu	Leu	Asp	Lys	Glu	Glu	Phe	Gln	Thr	Phe	Lys	20	25	30	35
Glu	Leu	Leu	Lys	Lys	Lys	Ser	Ser	Glu	Ser	Thr	Thr	Cys	Ser	Ile	40	45	50	55
Pro	Gln	Phe	Glu	Ile	Glu	Asn	Ala	Asn	Val	Glu	Cys	Leu	Ala	Leu	60	65	70	75
Leu	Leu	His	Glu	Tyr	Tyr	Gly	Ala	Ser	Leu	Ala	Trp	Ala	Thr	Ser	80	85	90	95
Ile	Ser	Ile	Phe	Glu	Asn	Met	Asn	Leu	Arg	Thr	Leu	Ser	Glu	Lys	100	105	110	115
Ala	Arg	Asp	Asp	Met	Lys	Asn	Ser	Pro	Glu	Asp	Pro	Glu	Ala	Thr	120	125	130	135
Met	Thr	Asp	Gln	Gly	Pro	Ser	Lys	Glu	Lys	Val	Pro	Glu	Asn	Lys	140	145	150	155
Tyr	Gly	Met	Thr	Lys	Leu	Ile	Leu	Gly	Val	Ser	Asp	Ile	Ser	Asp	160	165	170	175
Ser	Asn	Asn	Lys	His	Lys	Tyr	Val	Gly	Ile	His	Ser	Ser	Phe	Ala	180	185	190	195
Glu	Ile	Ser	Gln	Ala	Met	Glu	Gln	Glu	Gly	Ala	Thr	Ala	Ala	Glu	200	205	210	215
Thr	Glu	Glu	Gln	Glu	Ile	Ser	Gln	Ala	Met	Glu	Gln	Glu	Gly	Ala	220	225	230	235
Thr	Ala	Ala	Glu	Thr	Glu	Glu	Gln	Gly	His	Gly	Gly	Asp	Thr	Trp	240	245	250	255
Asp	Tyr	Lys	Ser	His	Val	Met	Thr	Lys	Phe	Ala	Glu	Glu	Glu	Asp	260	265	270	275
Val	Arg	Arg	Ser	Phe	Glu	Asn	Thr	Ala	Ala	Asp	Trp	Pro	Glu	Met	280	285	290	295
Gln	Thr	Leu	Ala	Gly	Ala	Phe	Asp	Ser	Asp	Arg	Trp	Gly	Phe	Arg	300	305	310	315
Pro	Arg	Thr	Val	Val	Leu	His	Gly	Lys	Ser	Gly	Ile	Gly	Lys	Ser	320	325	330	335
Ala	Leu	Ala	Arg	Arg	Ile	Val	Leu	Cys	Trp	Ala	Gln	Gly	Gly	Leu	340	345	350	355

	260		265		270
Tyr Gln Gly Met	Phe Ser Tyr Val Phe	Phe Leu Pro Val Arg	Glu		
	275		280		285
Met Gln Arg Lys	Lys Glu Ser Ser Val	Thr Glu Phe Ile Ser	Arg		
	290		295		300
Glu Trp Pro Asp	Ser Gln Ala Pro Val	Thr Glu Ile Met Ser	Arg		
	305		310		315
Pro Glu Arg Leu	Leu Phe Ile Ile Asp	Gly Phe Asp Asp Leu	Gly		
	320		325		330
Ser Val Leu Asn	Asn Asp Thr Lys Leu	Cys Lys Asp Trp Ala	Glu		
	335		340		345
Lys Gln Pro Pro	Phe Thr Leu Ile Arg	Ser Leu Leu Arg Lys	Val		
	350		355		360
Leu Leu Pro Glu	Ser Phe Leu Ile Val	Thr Val Arg Asp Val	Gly		
	365		370		375
Thr Glu Lys Leu	Lys Ser Glu Val Val	Ser Pro Arg Tyr Leu	Leu		
	380		385		390
Val Arg Gly Ile	Ser Gly Glu Gln Arg	Ile His Leu Leu Leu	Glu		
	395		400		405
Arg Gly Ile Gly	Glu His Gln Lys Thr	Gln Gly Leu Arg Ala	Ile		
	410		415		420
Met Asn Asn Arg	Glu Leu Leu Asp Gln	Cys Gln Val Pro Ala	Val		
	425		430		435
Gly Ser Leu Ile	Cys Val Ala Leu Gln	Leu Gln Asp Val Val	Gly		
	440		445		450
Glu Ser Val Ala	Pro Phe Asn Gln Thr	Leu Thr Gly Leu His	Ala		
	455		460		465
Ala Phe Val Phe	His Gln Leu Thr Pro	Arg Gly Val Val Arg	Arg		
	470		475		480
Cys Leu Asn Leu	Glu Glu Arg Val Val	Leu Lys Arg Phe Cys	Arg		
	485		490		495
Met Ala Val Glu	Gly Val Trp Asn Arg	Lys Ser Val Phe Asp	Gly		
	500		505		510
Asp Asp Leu Met	Val Gln Gly Leu Gly	Glu Ser Glu Leu Arg	Ala		
	515		520		525
Leu Phe His Met	Asn Ile Leu Leu Pro	Asp Ser His Cys Glu	Glu		
	530		535		540
Tyr Tyr Thr Phe	Phe His Leu Ser Leu	Gln Asp Phe Cys Ala	Ala		
	545		550		555
Leu Tyr Tyr Val	Leu Glu Gly Leu Glu	Ile Glu Pro Ala Leu	Cys		
	560		565		570
Pro Leu Tyr Val	Glu Lys Thr Lys Arg	Ser Met Glu Leu Lys	Gln		
	575		580		585
Ala Gly Phe His	Ile His Ser Leu Trp	Met Lys Arg Phe Leu	Phe		
	590		595		600
Gly Leu Val Ser	Glu Asp Val Arg Arg	Pro Leu Glu Val Leu	Leu		
	605		610		615
Gly Cys Pro Val	Pro Leu Gly Val Lys	Gln Lys Leu Leu His	Trp		
	620		625		630
Val Ser Leu Leu	Gly Gln Gln Pro Asn	Ala Thr Thr Pro Gly	Asp		
	635		640		645
Thr Leu Asp Ala	Phe His Cys Leu Phe	Glu Thr Gln Asp Lys	Glu		
	650		655		660
Phe Val Arg Leu	Ala Leu Asn Ser Phe	Gln Glu Val Trp Leu	Pro		
	665		670		675
Ile Asn Gln Asn	Leu Asp Leu Ile Ala	Ser Ser Phe Cys Leu	Gln		
	680		685		690
His Cys Pro Tyr	Leu Arg Lys Ile Arg	Val Asp Val Lys Gly	Ile		
	695		700		705
Phe Pro Arg Asp	Glu Ser Ala Glu Ala	Cys Pro Val Val Pro	Leu		
	710		715		720
Trp Met Arg Asp	Lys Thr Leu Ile Glu	Glu Gln Trp Glu Asp	Phe		
	725		730		735

Cys	Ser	Met	Leu	Gly	Thr	His	Pro	His	Leu	Arg	Gln	Leu	Asp	Leu	
				740					745					750	
Gly	Ser	Ser	Ile	Leu	Thr	Glu	Arg	Ala	Met	Lys	Thr	Leu	Cys	Ala	
				755					760					765	
Lys	Leu	Arg	His	Pro	Thr	Cys	Lys	Ile	Gln	Thr	Leu	Met	Phe	Arg	
				770					775					780	
Asn	Ala	Gln	Ile	Thr	Pro	Gly	Val	Gln	His	Leu	Trp	Arg	Ile	Val	
				785					790					795	
Met	Ala	Asn	Arg	Asn	Leu	Arg	Ser	Leu	Asn	Leu	Gly	Gly	Thr	His	
				800					805					810	
Leu	Lys	Glu	Glu	Asp	Val	Arg	Met	Ala	Cys	Glu	Ala	Leu	Lys	His	
				815					820					825	
Pro	Lys	Cys	Leu	Leu	Glu	Ser	Leu	Arg	Leu	Asp	Cys	Cys	Gly	Leu	
				830					835					840	
Thr	His	Ala	Cys	Tyr	Leu	Lys	Ile	Ser	Gln	Ile	Leu	Thr	Thr	Ser	
				845					850					855	
Pro	Ser	Leu	Lys	Ser	Leu	Ser	Leu	Ala	Gly	Asn	Lys	Val	Thr	Asp	
				860					865					870	
Gln	Gly	Val	Met	Pro	Leu	Ser	Asp	Ala	Leu	Arg	Val	Ser	Gln	Cys	
				875					880					885	
Ala	Leu	Gln	Lys	Leu	Ile	Leu	Glu	Asp	Cys	Gly	Ile	Thr	Ala	Thr	
				890					895					900	
Gly	Cys	Gln	Ser	Leu	Ala	Ser	Ala	Leu	Val	Ser	Asn	Arg	Ser	Leu	
				905					910					915	
Thr	His	Leu	Cys	Leu	Ser	Asn	Asn	Ser	Leu	Gly	Asn	Glu	Gly	Val	
				920					925					930	
Asn	Leu	Leu	Cys	Arg	Ser	Met	Arg	Leu	Pro	His	Cys	Ser	Leu	Gln	
				935					940					945	
Arg	Leu	Met	Leu	Asn	Gln	Cys	His	Leu	Asp	Thr	Ala	Gly	Cys	Gly	
				950					955					960	
Phe	Leu	Ala	Leu	Ala	Leu	Met	Gly	Asn	Ser	Trp	Leu	Thr	His	Leu	
				965					970					975	
Ser	Leu	Ser	Met	Asn	Pro	Val	Glu	Asp	Asn	Gly	Val	Lys	Leu	Leu	
				980					985					990	
Cys	Glu	Val	Met	Arg	Glu	Pro	Ser	Cys	His	Leu	Gln	Asp	Leu	Glu	
				995					1000					1005	
Leu	Val	Lys	Cys	His	Leu	Thr	Ala	Ala	Cys	Cys	Glu	Ser	Leu	Ser	
				1010					1015					1020	
Cys	Val	Ile	Ser	Arg	Ser	Arg	His	Leu	Lys	Ser	Leu	Asp	Leu	Thr	
				1025					1030					1035	
Asp	Asn	Ala	Leu	Gly	Asp	Gly	Gly	Val	Ala	Ala	Leu	Cys	Glu	Gly	
				1040					1045					1050	
Leu	Lys	Gln	Lys	Asn	Ser	Val	Leu	Thr	Arg	Leu	Gly	Leu	Lys	Ala	
				1055					1060					1065	
Cys	Gly	Leu	Thr	Ser	Asp	Cys	Cys	Glu	Ala	Leu	Ser	Leu	Ala	Leu	
				1070					1075					1080	
Ser	Cys	Asn	Arg	His	Leu	Thr	Ser	Leu	Asn	Leu	Val	Gln	Asn	Asn	
				1085					1090					1095	
Phe	Ser	Pro	Lys	Gly	Met	Met	Lys	Leu	Cys	Ser	Ala	Phe	Ala	Cys	
				1100					1105					1110	
Pro	Thr	Ser	Asn	Leu	Gln	Ile	Ile	Gly	Leu	Trp	Lys	Trp	Gln	Tyr	
				1115					1120					1125	
Pro	Val	Gln	Ile	Arg	Lys	Leu	Leu	Glu	Glu	Val	Gln	Leu	Leu	Lys	
				1130					1135					1140	
Pro	Arg	Val	Val	Ile	Asp	Gly	Ser	Trp	His	Ser	Phe	Asp	Glu	Asp	
				1145					1150					1155	
Asp	Arg	Tyr	Trp	Trp	Lys	Asn									
				1160											

<210> 2

<211> 542

<212> PRT

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 7477736CD1

<400> 2

Met	Ala	Leu	Pro	Tyr	Cys	Ile	Phe	Leu	Phe	Thr	Val	Leu	Ser	Pro
1				5					10					15
Pro	Phe	Ser	Leu	Thr	Ala	Pro	Ser	Pro	Cys	His	Cys	Arg	Thr	Ser
				20					25					30
Ser	Ser	Pro	Tyr	Gln	Ala	Phe	Leu	Trp	Arg	Met	Arg	Arg	Pro	Arg
				35					40					45
His	Ile	Asp	Ala	Pro	Ser	Tyr	Arg	Ser	Leu	Ser	Lys	Gly	Asn	Pro
				50					55					60
Ala	Phe	Thr	Ala	His	Thr	His	Met	Pro	His	Asn	Cys	Tyr	Asn	Ser
				65					70					75
Ala	Thr	Leu	Cys	Met	His	Ala	Asn	Thr	His	Tyr	Trp	Thr	Gly	Lys
				80					85					90
Met	Ile	Asn	Pro	Ser	Cys	Pro	Gly	Gly	Leu	Gly	Ala	Thr	Ile	Cys
				95					100					105
Trp	Thr	Tyr	Phe	Thr	His	Thr	Gly	Met	Ser	Asp	Gly	Gly	Gly	Val
				110					115					120
Gln	Asp	Gln	Ala	Arg	Glu	Lys	His	Val	Lys	Glu	Val	Ile	Ser	Gln
				125					130					135
Leu	Thr	Arg	Val	His	Ser	Thr	Pro	Ser	Pro	Tyr	Lys	Gly	Leu	Asp
				140					145					150
Leu	Ser	Lys	Leu	His	Glu	Thr	Leu	Arg	Thr	His	Thr	His	Leu	Val
				155					160					165
Ser	Leu	Phe	Asn	Thr	Thr	Leu	Thr	Gly	Leu	His	Glu	Val	Ser	Ala
				170					175					180
Gln	Asn	Pro	Thr	Asn	Cys	Trp	Met	Cys	Leu	Pro	Leu	His	Phe	Arg
				185					190					195
Pro	Tyr	Ile	Ser	Val	Pro	Val	Pro	Glu	Gln	Trp	Asn	Asn	Phe	Ser
				200					205					210
Thr	Glu	Ile	Asn	Thr	Thr	Ser	Ile	Leu	Val	Gly	Pro	Leu	Val	Ser
				215					220					225
Asn	Leu	Glu	Ile	Ile	His	Thr	Ser	Asn	Leu	Thr	Cys	Val	Lys	Phe
				230					235					240
Ser	Asn	Thr	Ile	Ala	Thr	Thr	Asn	Ser	Gln	Cys	Ile	Arg	Trp	Val
				245					250					255
Thr	Pro	Pro	Thr	Gln	Ile	Val	Cys	Leu	Pro	Ser	Gly	Ile	Phe	Phe
				260					265					270
Val	Cys	Gly	Thr	Ser	Ala	Tyr	His	Cys	Leu	Asn	Gly	Ser	Ser	Glu
				275					280					285
Ser	Met	Cys	Phe	Leu	Ser	Phe	Leu	Val	Pro	Pro	Met	Thr	Ile	Tyr
				290					295					300
Thr	Glu	Gln	Asp	Leu	Tyr	Asn	His	Val	Val	Pro	Lys	Pro	His	Asn
				305					310					315
Lys	Arg	Val	Pro	Ile	Leu	Pro	Phe	Val	Ile	Arg	Ala	Gly	Val	Leu
				320					325					330
Gly	Arg	Leu	Gly	Thr	Gly	Ile	Gly	Ser	Ile	Thr	Thr	Ser	Thr	Gln
				335					340					345
Phe	Tyr	Tyr	Lys	Leu	Ser	Gln	Glu	Ile	Asn	Gly	Asp	Met	Glu	Gln
				350					355					360
Val	Thr	Asp	Ser	Leu	Val	Thr	Leu	Gln	Asp	Gln	Leu	Asn	Ser	Leu
				365					370					375
Ala	Ala	Val	Val	Leu	Gln	Asn	Arg	Arg	Ala	Leu	Asp	Leu	Leu	Thr
				380					385					390
Ala	Lys	Arg	Gly	Gly	Thr	Cys	Leu	Phe	Leu	Gly	Glu	Glu	Cys	Cys
				395					400					405
Tyr	Tyr	Val	Asn	Gln	Ser	Arg	Ile	Val	Thr	Glu	Lys	Val	Lys	Glu
				410					415					420
Ile	Arg	Asp	Arg	Ile	Gln	Cys	Arg	Ala	Glu	Glu	Leu	Gln	Asn	Thr
				425					430					435

Glu	His	Trp	Gly	Leu	Leu	Ser	Gln	Trp	Met	Pro	Trp	Val	Leu	Pro
			440						445					450
Phe	Leu	Gly	Pro	Leu	Ala	Ala	Leu	Ile	Leu	Leu	Leu	Phe	Gly	
			455						460					465
Pro	Cys	Ile	Phe	Asn	Leu	Leu	Val	Lys	Phe	Val	Ser	Ser	Arg	Ile
			470						475					480
Glu	Ala	Val	Lys	Leu	Gln	Met	Val	Leu	Gln	Met	Glu	Pro	Gln	Met
			485						490					495
Gln	Ser	Met	Thr	Lys	Ile	Tyr	His	Gly	Pro	Leu	Asp	Arg	Pro	Ala
			500						505					510
Ser	Pro	Cys	Ser	Asp	Val	Asn	Asp	Ile	Glu	Gly	Thr	Pro	Pro	Lys
			515						520					525
Glu	Ile	Ser	Thr	Ala	Gln	Pro	Leu	Leu	Cys	Pro	Asn	Ser	Ala	Gly
			530						535					540
Ser	Ser													

<210> 3

<211> 3489

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 7474830CB1

<400> 3

```

atggaaggag acaaatcgct caccttttcc agctacgggc tgcaatgggtg tctctatgag 60
ctagacaagg aagaatttca gacattcaag gaattactaa agaagaaatc ttcagaatcg 120
accacatgct ctattccaca gtttgaaatc gagaatgcca acgtggaatg tctggcactc 180
ctcttgcatg agtattatgg agcatcgctg gcctgggcta cgtccattag catctttgaa 240
aacatgaacc tgcgaaccct ctcgagaag gcacgggatg acatgaaaaa ttcaccagaa 300
gatcctgaag caacgatgac tgaccaagga ccaagcaagg aaaaagtgcc agaaaataaa 360
tatggcatga ctaagcttat cttgggggtg tctgacatct ctgactcgaa taataaacac 420
aagtatgttg gaattcattc ttcttttgca gaaatttcac aagctatgga acaagaaggt 480
gccacagcag cacagacaga agaacaagaa atttcacaag ctatggaaca agaaggtgcc 540
acagcagcag agacagaaga acaaggacat ggaggtgaca catgggacta caagagtcac 600
gtgatgacca aattcgctga ggaggaggat gtacgtcgta gttttgaaaa cactgctgct 660
gactggccgg aaatgcaaac gttggctggg gcttttgatt cagaccgggtg gggcttccgg 720
cctcgcacgg tggttctgca cggaaagtca ggaattggga aatcggctct agccagaagg 780
atcgtgctgt gctgggcgca aggtggactc taccagggaa tgttctccta cgtcttcttc 840
ctccccgtta gagagatgca tcggaagaag gagagcagtg tcacagagtt catctccagg 900
gagtgccag actcccaggc tccgggtgacg gagatcatgt cccgaccaga aaggctgttg 960
ttcatcattg acggtttcga tgacctgggc tctgtcctca acaatgacac aaagctctgc 1020
aaagactggg ctgagaagca gcctccgttc accctcatac gcagtctgct gaggaaggtc 1080
ctgctccctg agtcccttct gatcgtcacc gtcagagacg tgggcacaga gaagctcaag 1140
tcagaggtcg tgtctccccg ttacctgtta gttagaggaa tctccgggga acaaagaatc 1200
cacttgctcc ttgagcgcgg gattggtgag catcagaaga cacaagggtt gcgtgcatc 1260
atgaacaacc gtgagctgct cgaccagtgc cagggtgccg ccgtgggctc tctcatctgc 1320
gtggccctgc agctgcagga cgtggtgggg gagagcgtcg ccccttcaa ccaaagctc 1380
acaggcctgc acgccgcttt tgtgtttcat cagctcacc ctcgaggcgt ggtccggcgc 1440
tgtctcaatc tggaggaaag agttgtcctg aagcgcttct gccgtatggc tgtggaggga 1500
gtgtggaata ggaagtcagt gtttgacggt gacgacctca tgggtcaagg actcggggag 1560
tctgagctcc gtgctctgtt tcacatgaac atccttctcc cagacagcca ctgtgaggag 1620
tactacacct tcttcacct cagtctccag gacttctgtg ccgcttcta ctacgtgtta 1680
gagggcctgg aaatcgagcc agctctctgc cctctgtacg ttgagaagac aaagaggtcc 1740
atggagctta aacaggcagg cttccatata cactcgcttt ggatgaagcg tttcttgttt 1800
ggcctcgtga gcgaagacgt aaggaggcca ctggaggtcc tgcctggcgt tcccgttccc 1860
ctgggggtga agcagaagct tctgcaactg gtctctctgt tgggtcagca gcctaatagcc 1920
accaccccag gagacacctt ggacgccttc cactgtcttt tcgagactca agacaaagag 1980
tttgttcgct tggcattaaa cagcttccaa gaagtgtggc ttccgattaa ccagaacctg 2040
gacttgatag catcttctct ctgcctccag cactgtccgt atttgcgga aattcgggtg 2100
gatgtcaaag ggtatcttccc aagagatgag tccgctgagg catgtcctgt ggtccctcta 2160

```

```

tggatgcggg ataagacctt cattgaggag cagtgggaag atttctgctc catgcttggc 2220
accacccac acctgcggca gctggacctg ggcagcagca tcttgacaga gcgggccatg 2280
aagacctgt gtgccaagct gaggcatccc acctgcaaga tacagacctt gatgtttaga 2340
aatgcacaga ttacctctgg tgtgcagcac ctctggagaa tcgtcatggc caaccgtaac 2400
ctaagatccc tcaacttggg aggcacccac ctgaaggaa aggatgtaag gatggcgtgt 2460
gaagccttaa aacacccaaa atgtttgttg gagtctttga ggctggattg ctgtggattg 2520
acccatgcct gttacctgaa gatctcccaa atccttacga cctccccag cctgaaatct 2580
ctgagcctgg caggaaacaa ggtgacagac cagggagtaa tgctctcag tgatgccttg 2640
agagtctccc agtgcgccc gcagaagctg atactggagg actgtggcat cacagccagc 2700
ggttgccaga gtcctggctc agccctcgct agcaaccgga gcttgacaca cctgtgccta 2760
tccaacaaca gcctggggaa cgaagggtga aatctactgt gtcgatccat gaggcttccc 2820
cactgtagtc tgcagaggct gatgctgaat cagtgccacc tggacacggc tggctgtggt 2880
tttcttgac ttgcgcttat gggtaactca tggctgacgc acctgagcct tagcatgaac 2940
cctgtggaag acaatggcgt gaagcttctg tgcgagggtca tgagagaacc atcttgtcat 3000
ctccaggacc tggagttggt aaagtgtcat ctacccgccg cgtgctgtga gagtctgtcc 3060
tgtgtgatct cgaggagcag acacctgaag agcctggatc tcacggacaa tgccctgggt 3120
gacggtgggg ttgctgcact gtgcgaggga ctgaagcaaa agaacagtgt tctgacgaga 3180
ctcgggttga aggcattgtg actgacttct gattgctgtg aggcactctc cttggccctt 3240
tctgcaacc ggcattctgac cagtctaaac ctggtgcaga ataacttcag tcccaaagga 3300
atgatgaagc tgtgttcggc ctttgcctgt cccacgtcta acttacagat aattgggctg 3360
tggaatggc agtaccctgt gcaaatagg agactgtctg aggaagtga gctactcaag 3420
ccccgagtcg taattgacgg tagttggcat tcttttgatg aagatgaccg gtactggtgg 3480
aaaaactga                                     3489

```

<210> 4

<211> 2074

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<223> Incyte ID No: 747736CB1

<220>

<221> unsure

<222> 1995

<223> a, t, c, g, or other

<400> 4

```

gaggatctgt gctgtctctt caagcaacaa ccgtgaggaa agtaactaaa atcgtaaattc 60
cccatggccc tcccttattg tttttttctc tttactgttc tctcaccacc tttcagtctc 120
actgcacctt ctccatgcca ctgtaggacc agtagctccc cttaccaagc gtttctatgg 180
agaatgcggc gtcccagaca tattgatgcc ccacgtata ggagtttatc taagggaaac 240
ccgccttca ccgccacac ccatatgccc cacaactgct ataactctgc cactctttgt 300
atgcatgcaa atactcatta ttggacaggg aaaatgatta atcctagtgt tcttgaggga 360
cttggagcca ctatctgttg gacttacttc accataccg gtatgtctga tgggggtgga 420
gttcaagatc aggcaagaga aaaacatgta aaggaagtaa tctcccaact caccgggta 480
catagcacc ctagcccta caaaggacta gatctctcaa aactacatga aaccctccgt 540
accatactc acctggtaag cctatttaat accacctca ctgggctcca tgagggtctc 600
gccaaaacc ctactaactg ttggatgtgc ctccccctgc acttcaggcc atacatttca 660
gtccctgtac ctgaacaatg gaataacttc agcacagaaa taaacaccac ttccatttta 720
gtaggacctc ttgtttccaa tctggaaata atccatacct caaacctcac ctgtgtaaaa 780
tttagcaata ctatagcaac aaccaactcc cagtgcacat gatgggtaac tctccaca 840
caaatagctt gcctaccctc aggaatatct tttgtctgtg gtacctcagc ctatcattgt 900
ttgaatggct cttcagaatc tatgtgtctc ctctcattct tagtgcccc tatgaccatc 960
tacactgaac aagatttata caatcatgtc gtacctaaag cccacaacaa aagagtacc 1020
attcttccct ttgtttcagc agcaggagtg ctaggcagac taggtactgg cattggcagt 1080
atcacacct ctactcagtt ctactacaaa ctatctcaag aaataaatgg tgacatggaa 1140
caggtcactg actcctggt cacttgcaa gatcaactta actccctagc agcagtagtc 1200
cttcaaaatc gaagagcttt agacttgcta accgcaaaa gagggggaac ctgtttatct 1260
ttaggagaag aatgctgtta ttatgttaat caatccagaa ttgtcactga gaaagttaa 1320
gaaattcgag atcgaaatac atgtagagca gaggagcttc aaaacaccga acactggggt 1380
ctcctcagcc aatggatgcc ctgggttctc cccttcttag gacctctagc agctctaata 1440

```

ttgttactcc	tctttggacc	ctgtatcttt	aacctccttg	ttaagtttgt	ctcttcaga	1500
attgaagctg	taaagctaca	aatggttctt	caaattggagc	cccagatgca	gtccatgact	1560
aaaatctacc	acggacccct	ggaccggcct	gctagcccat	gtccgatgt	taatgacatc	1620
gaaggcactc	ctccaagga	aatctcaact	gcacaacccc	tactatgcc	caattcagca	1680
ggaagcagtt	agagcggctg	tcagtcaacc	tcccaacag	cacttgggtt	ttcctgttga	1740
gaggggggac	tgagagacag	gactagctgg	atttcctagg	cggattaaga	atccctaagc	1800
ctagctggga	aggtgaccgc	gtccaccttt	aaacacgggg	cttgcaactt	agctcacacc	1860
caaccaatca	gagagctcac	taaaatgcta	attaggcaaa	aacaggaggt	aaagaaatag	1920
ccaatcatct	attgcctgag	agcacagtgg	gagggacaag	gattgcaata	taaaccagg	1980
cattcgagcc	agcanagcaa	ccgcctttgg	gtcccttccc	cttgatggg	agctctgttt	2040
tcactctatt	tcactctatt	aaatcttgca	actg			2074